PD1/PDL1 Expression and Molecular Associations in HPB Malignancies

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Background

Cholangiocarcinoma (CC), hepatocellular carcinoma (HCC), and pancreatic ductal adenocarcinoma (PDAC) are all devastating malignancies. Agents targeting the PD1 (programmed death 1) immune checkpoint pathway (pembrolizumab, nivolumab) have been shown to improve survival in lung, melanoma, bladder and renal cell carcinoma. Expression of the PD1 ligand (PD-L1) in tumor cells has demonstrated utility in predicting improved responses to these agents in some studies, and a PDL1 companion diagnostic has been recently FDA-approved for NSCLC. Immune checkpoint inhibitors are actively being explored for their efficacy in various solid tumors, including hepato-pancreatico-biliary (HPB) cancers. Limited data exists around PD-1 and PDL1 expression in these cancers, therefore we assessed expression of PD1/PDL1 in a large cohort of patients with HPB cancers and explored the existence of accompanying genomic mutations associated with expression of PD-1/PD-L1.

Methods

524 patients with HPB cancers (354 PDAC, 58 Hepatocellular carcinoma (HCC), 54 intrahepatic (IhCC), 18 extrahepatic (EhCC), and 40 gallbladder (GBC)) were included in the study and tumors tested centrally at a CLIA lab (Caris Life Sciences, Phoenix, AZ). Tests included one or more of the following: gene sequencing (next generation sequencing, Illumina TruSeq), protein expression (immunohistochemistry [IHC]) and gene amplification (in-situ hybridization [ISH]). PD-1 (MRQ22 antibody;≥1+ staining of tumor infiltrating lymphocytes [TILs] was used as a cutoff) and PD-L1 (SP142 antibody; ≥2+≥5% staining in tumor cells was used as a cutoff) status was tested in all samples. Two-tailed Fisher's exact test was performed to test where proportions of positive results were different by subgroup (p≤0.05).

Table 1. Clinicopathologic Parameters

		Stage		Gender		Age	
	Total (n)	Metastatic	Localized	Male	Female	Median	Range
Pancreatic	354	229 (65%)	125 (35%)	195 (55%)	159 (45%)	64	30-88
GBC	40	29 (73%)	11 (28%)	18 (45%	22 (55%)	60	32-82
EhCC	18	13 (72%)	5 (28%)	10 (56%)	8 (44%)	58	33-79
IhCC	54	46 (85%)	8 (15%)	27 (50%	27 (50%)	60	36-85
HCC	58	22 (38%)	36 (62%)	44 (76%)	14 (24%)	60	17-87
Total	524						

Figure 1. Sites of Specimen Collection

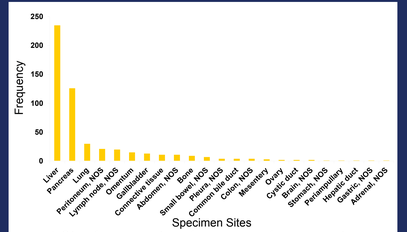
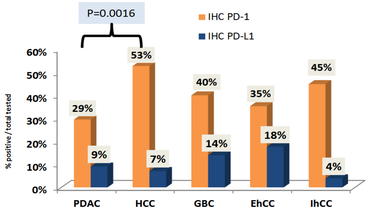


Figure 1. Sites of specimens utilized for molecular profiling. The most common specimen sites for metastatic cases were: liver for pancreatic cancers, liver for GBC, liver for EhHC, liver for IhCC and lung for Liver HCC.

Results

- Among those with PDAC, HCC, IhCC, EhCC, and GBC, rates of PD1 expression on tumor infiltrating lymphocytes (TILs) and PD-L1 expression on tumor cells are detailed in Figure 2.
- In PD-L1 positive, compared with PD-L1 negative HPB cancers, mutations in the following were more prevalent, though not statistically significant: BRCA2, ATM, SMAD4 and PTEN.
- Among all theranostic biomarkers tested, TOP2A expression (IHC) was significantly increased in PD-L1+ versus PD-L1- tumors (82% vs. 60%; p=0.0083).

Figure 2: Rates of PD-1 and PD-L1 Positivity in HPB Malignancies



	PDAC	HCC	GBC	EhCC	IhCC
PD1 (n)	295	55	35	17	49
PDL1 (n)	309	56	35	17	51

Figure 2. Percent frequencies of PD1 positive tumor infiltrating lymphocytes and PDL1 expression in tumor cells across HPB malignancies.

Figure 3. Mutations across HPB Malignancies

Figure 3. Mutation rates of genes with variants detected by next generation sequencing. Most frequently mutated genes across HPB malignancies were TP53, followed by KRAS.

Figure 4. Comparison of IHC/ISH Theranostic Biomarkers in PDL1+ vs. PDL1- HPB Malignancies

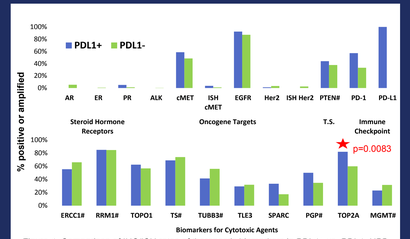


Figure 4. Comparison of IHC/ISH rates of theranostic biomarkers in PDL1+ vs. PDL1- HPB malignancies. IHC biomarkers are represented as percent positive frequency unless indicated with # which reflects percent negative frequency. Amplification rates also provided for cMET and HER2. TOP2A positivity was associated with PDL1 positive HPB malignancies.

Figure 5. Mutation frequencies in PDL1+ vs. PDL1 – HPB Malignancies

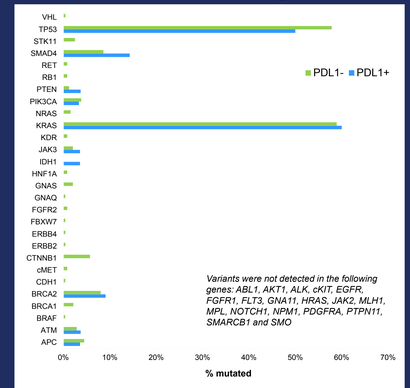


Figure 5. Comparison of mutation rates in PDL1 + vs. PDL1- HPB malignancies. Rates were not significantly different among the two groups.

Figure 6. Representative IHC staining patterns

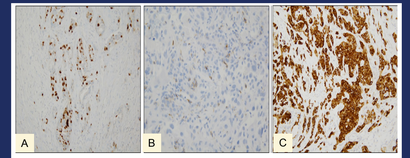


Figure 6. Representative immunohistochemistry staining patterns. 6A. 20x TP53-mutated cholangiocarcinoma with TOP2A positive expression (2+ 30%). 6B. 40x KRAS wildtype pancreatic cancer with PD1-positive tumor infiltrating lymphocytes. 6C. 20x KRAS-mutated pancreatic cancer with PDL1 positive expression in tumor tissue (2+80%).

Conclusions

- HPB tumors express PD-L1 at a frequency of 4-18%, and PD-1 at a frequency of 29-45%. PDL1 expression suggests a potential role of immune checkpoint inhibitors in these cancers.
- A statistically significant association of mutations with PD-1+ or PD-L1+ tumors was not identified in this group of tumors.
- PD-L1 expression associates with TOP2A expression, a marker of proliferation and also anthracycline sensitivity. Further evaluation of the biological significance of this correlation and PDL1/PD-1 + anthracycline combination therapy may be warranted.

References

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